

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 2

PATENT
Attorney Docket No.: JHU1630

In the Specification

Please amend the paragraph beginning at page 32, line 19 as follows:

Cell Lines and Tissues. The breast cancer cell lines MDAMB435, MCF7, T47D, SKBR3, ZR75.1, MDAMB468, HS578T, MDAMB231 and the immortal human mammary epithelial cell lines (HMEC) MCF10A and HBL100 were obtained and maintained in culture according to instructions (ATCC, Rockville, MD). The two matched tumor cell lines, 21PT, derived from a primary tumor and 21MT, from the metastasis of the same patient, were propagated as described elsewhere. The breast cancer cell line, MW, was obtained from Dr. Renato Dulbecco. HMEC-H16N (immortalized with HPV) was kindly provided by Dr. Vimla Band. Cultured finite life span human breast epithelial cell strains 04372, 219-6, and 166372 were obtained from Clonetics (Walkersville, MD), and HMEC strains 1-26 and 3-14 were kindly provided by Dr. Steve Ethier. Finite life span HMEC 184, the immortalized HMECs 184A1 (passage 15 and 99) and 184B5 were kindly provided by Dr. Martha Stampfer, and grown as described (~~<http://www.lbl.gov/LBL-Programs/mrgs/review.html>~~) on the world-wide web at address [lbl.gov/LBL-Programs/mrgs/review](http://www.lbl.gov/LBL-Programs/mrgs/review). Cell extracts from finite lifespan HMECs 70N and 81N were kindly provided by Dr. Khandan Keyomarsi. Mammary organoids were prepared from reduction mammoplasty specimens of women with benign or no abnormalities in the breast following collagenase digestion as described in Bergstraesser LM, (1993). Human mammary luminal and myoepithelial cells were prepared by progressive collagenase digestion of breast tissue, sedimentated to obtain organoids (ductal and lobulo-alveolar fragments), cultured short term, and finally highly enriched by using an immunomagnetic separation technique (Niranjan B, 1995).

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 3

PATENT
Attorney Docket No.: JHU1630

In the claims

Please cancel claims 2-6, 8, 15-17, 19, 26-29, and 33-37 without prejudice.

Please amend claims 1, 7, 9-12, 14, 18, 21, 22, 25, and 30-32 as follows:

1. (Currently Amended) A method of diagnosing ~~a cellular proliferative disorder of breast tissue~~ breast cancer or ductal carcinomas *in situ* (DCIS) in a subject comprising determining the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids isolated from a sample comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject, wherein ~~[[the]]~~ a state of hypermethylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids as compared with the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids in comparable samples obtained from a subject not having the cellular proliferative disorder of breast tissue normal subjects is indicative of ~~a cellular proliferative disorder of breast tissue~~ breast cancer breast cancer or DCIS in the subject.

Claims 2-6. (Cancelled)

7. (Currently Amended) The method of claim ~~[[6]]~~ 1, wherein the duct cells are obtained by a procedure selected from ductal lavage, sentinel node biopsy, fine needle aspirate, routine operative breast endoscopy, nipple aspiration and core biopsy.

Claim 8. (Cancelled)

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 4

PATENT
Attorney Docket No.: JHU1630

9. (Currently Amended) The method of claim ~~[[2]]~~ 1, wherein determining the state of methylation comprises amplifying the nucleic acid by means of at least one sense primer and at least one antisense primer that distinguishes between methylated and unmethylated nucleic acids.
10. (Currently Amended) The method of claim 9, wherein the primers hybridize with target polynucleotide sequences selected from SEQ ID NO:~~1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82~~ and combinations thereof.
11. (Currently Amended) The method of claim 9, wherein the primers are selected from SEQ ID NO:~~7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90~~ and combinations thereof.
12. (Currently Amended) The method of claim ~~[[2]]~~ 1, further comprising contacting the nucleic acid with a methylation-sensitive restriction endonuclease.
13. (Original) The method of claim 12, wherein the methylation-sensitive restriction endonuclease is selected from the group consisting of MspI, HpaII, BssHII, BstUI and NotI.

14. (Currently Amended) A method of determining a predisposition to ~~a cellular proliferative disorder of breast tissue~~ breast cancer or DCIS in a subject comprising determining the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids isolated from a sample comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject,
- ~~wherein the nucleic acid is selected from the group consisting of Twist, cyclin D2, RAR β 2, HOXA5, WT1, 14.3.3 sigma, estrogen receptor, NES-1 and combinations thereof; and~~
- wherein ~~[[the]]~~ a state of hypermethylation of the nucleic acid(s) as compared with the state of methylation of CpG islands in the promoter of cyclin D2 nucleic acids in comparable samples obtained from ~~a subject not having the~~ normal subjects is indicative of a ~~cellular proliferative disorder of breast tissue~~ breast cancer or DCIS in the subject.
- 15-17. Cancelled
18. (Currently Amended) The method of claim ~~[[17]]~~ 14, wherein the duct cells are obtained by a procedure selected from the group consisting of ductal lavage, sentinel node biopsy, fine needle aspirate, routine operative breast endoscopy, nipple aspiration and core biopsy.
19. (Cancelled)
20. (Original) The method of claim 14, wherein determining the state of methylation comprises amplifying the nucleic acid(s) by means of at least one sense primer and at least one antisense primer that distinguishes between methylated and unmethylated nucleic acid.

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 6

PATENT
Attorney Docket No.: JHU1630

21. (Currently Amended) The method of claim 20, wherein the primers hybridizes with target polynucleotide sequences selected from SEQ ID NO:~~1-4~~, 15-18, ~~25-36~~, ~~41-48~~, ~~65-66~~, ~~73-76~~, ~~81-82~~, and combinations thereof.
22. (Currently Amended) The method of claim 20, wherein the primers are selected from SEQ ID NO:~~7-14~~, 21-24, ~~37-40~~, ~~49-64~~, ~~69-72~~, ~~77-80~~, ~~85-90~~ and combinations thereof.
23. (Original) The method of claim 14, further comprising contacting the nucleic acid with a methylation-sensitive restriction endonuclease.
24. (Original) The method of claim 23, wherein the methylation-sensitive restriction endonuclease is selected from the group consisting of MspI, HpaII, BssHII, BstUI and NotI.

25. (Currently Amended) A method for diagnosing a cellular proliferative disorder of breast tissue in a subject comprising:

- (a) contacting a nucleic acid-containing specimen comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof, from the subject with an agent that provides a determination of the methylation state of CpG islands in the promoter of cyclin D2 nucleic acids in the specimen, and
- (b) identifying the methylation state of at least one ~~region of at least one~~ CpG island in the promoter of cyclin D2 nucleic acid, wherein ~~[[the]]~~ hypermethylation state of the at least one ~~region of at least one~~ CpG island in the promoter of cyclin D2 nucleic acid ~~different from~~ compared with the methylation state of ~~the same~~ region CpG islands in the promoter of cyclin D2 nucleic acids in comparable samples obtained from a subject not having the cellular proliferative disorder of breast tissue normal subjects is indicative of a ~~cellular proliferative disorder of breast tissue~~ breast cancer or DCIS in the subject.

Claims 26-29 (Cancelled)

- 30. (Currently Amended) The method of claim ~~[[27]]~~ 25, wherein the agent is at least one sense primer and at least one ~~antisense~~ antisense primer that hybridizes with a target sequence in the nucleic acid.
- 31. (Currently Amended) The method of claim 30, wherein the target nucleic acid sequence is selected from ~~SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82, and~~ combinations thereof.

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 8

PATENT
Attorney Docket No.: JHU1630

32. (Currently Amended) The method of claim 30, wherein the primers are selected from the group consisting of SEQ ID NO:7-14, 21-24, ~~37-40, 49-64, 69-72, 77-80, 85-90~~ and combinations thereof.

Claims 33-34 (Cancelled)

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 9

PATENT
Attorney Docket No.: JHU1630

REMARKS

Claims 1-37 were pending prior to this Response, with claims 35-37 being withdrawn as subject to a restriction requirement. By the present communication, the paragraph beginning at page 32, line 19 has been amended to delete an active hyperlink. In the claims, no claims have been added, claims 2-6, 9, 25-27, 29, 26-29 and 33-37 have been cancelled without prejudice, and claims 1, 7, 9-12, 14, 18, 21, 22, 25, and 30-32 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1, 7, 9-14, 18, 20-25 and 30-32 are currently pending in this application.

The Objection to the Specification

The Office Action contains an objection to the Specification for containing an embedded hyperlink and/or other form of browser-executable code. To overcome the objection, by the present communication Applicants have amended the paragraph beginning at line 19 of page 32 to remove the active hyperlink.

In addition, the Examiner has requested that either the drawings or the description of the drawings be amended to set forth the proper sequence identifiers for each sequence. In response to this objection to the Specification, Applicants submit herewith copies of Figures 1-8 with mark-ups showing proposed amendments to Figures to recite the sequence identifiers for the sequences shown in the Figures. Applicant will provide formal drawings that include corresponding amendments to Figures 1-8 upon allowance of claims in this application.

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 10

PATENT
Attorney Docket No.: JHU1630

In view of the amendment and the marked-up copies of Figures 1-8 showing proposed amendments to add proper sequence identifiers, Applicants respectfully request reconsideration and withdrawal of the objection to the Specification.

The Rejection under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 1-34 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. Applicants disagree with the Examiner's assertion that the Specification fails to provide sufficient description to enable those of skill in the art to make or use the invention commensurate in scope with the previously presented claims. By the present communication, previously presented claims 2-6, 8, 15-17, 19, 26-29 and 33-34 have been cancelled without prejudice, rendering the rejection moot as to the subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 7, 9-14, 18, 20—25 and 30-32.

The Examiner asserts that the claims do not set forth the relationship between the nucleic acids of a subject and “the same” nucleic acids of a control that is not a comparison of nucleic acids between two individuals. To address the Examiner's concern, Applicants have amended claims 1, 16 and 28 to require detection of a state of hypermethylation in the patient's nucleic acid as compared with “the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids in comparable samples obtained from normal subjects.” Thus, the “control” is not limited to breast tissue of a single normal individual, but is required to be representative of nucleic acids obtained from a plurality of normal, i.e., the absence of hypermethylation in CpG islands in the promoter of cyclin D2 nucleic acids in a broad population of normal subjects.

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 11

PATENT
Attorney Docket No.: JHU1630

Further, the Examiner asserts that there is no teaching or guidance in the specification that hypermethylation in an intron or exon of cyclin D2 would lead to decreased expression of cyclin D2 or be associated with breast cancer or any cellular proliferative disorder of the breast, thus causing those of skill in the art to allegedly engage in undue experimentation to practice the invention. However, the invention methods for detecting breast cancer or DCIS, as recited amended claims 1, 14 and 25, require detection of a state of hypermethylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids in the subject's sample as an indication that the subject has primary breast cancer. Thus, hypermethylation in an intron or exon of cyclin D2 is excluded by the claim amendments.

The Examiner acknowledges that the Specification is enabling for an embodiment of the invention described as follows: "a method of detecting breast cancer or DCIS in a subject comprising obtaining nucleic acid from a blood, plasma, lymph, duct cells ductal lavage fluid, nipple aspiration fluid, breast tissue lymph nodes or bone marrow specimen of a subject and determining the state of methylation of CpG islands of the promoter of cyclin D2 nucleic acids, wherein hypermethylation of CpG islands in the promoter of cyclin D2 is indicative of breast cancer in the subject" (Office Action, page 3). To reduce the issues and expedite prosecution, Applicants have amended independent claims 1, 14 and 25 to focus the invention on the subject matter that the Examiner has indicated is allowable. However, Applicants specifically reserve the right to pursue other embodiments of the invention in a subsequently filed application.

In view of the amendments and for the reasons discussed above, Applicants submit that the Examiner's concern that those of skill in the art would have to engage in undue experimentation in order to practice the claimed invention has been overcome. Accordingly, reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 112, first paragraph, are respectfully requested.

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 12

PATENT
Attorney Docket No.: JHU1630

The Rejection under 35 U.S.C. § 102(a)

A. Applicants respectfully traverse the rejection of claims 1, 25-27, 30, 33 and 34 under 35 U.S.C. § 102(a) as allegedly being anticipated by Ferguson et al. (*PNAS* 97:6049-6054, 2000; hereinafter "Ferguson"). By the present communication, previously presented claims 27, 33 and 34 have been cancelled without prejudice, rendering the rejection moot as to the subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 25, 26 and 30.

Applicants submit that the invention methods for detecting primary breast cancer in a subject, as defined by amended claims 1, 25, distinguish over the disclosure of Ferguson by requiring:

determining the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids isolated from a sample or specimen comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject, wherein a state of hypermethylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids as compared with the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids in comparable samples obtained from normal subjects is indicative of breast cancer or DCIS in the subject.

By contrast, Ferguson is absolutely silent regarding all elements of the invention methods for detecting breast cancer or DCIS as defined by amended claim 1 and 25. The Examiner asserts that Ferguson discloses that hypermethylation of the sigma promoter, for example, a CpG rich region of the 14.3.3 sigma gene promoter, is largely responsible for silencing of the sigma gene and occurs in a majority breast cancers. However, Applicants respectfully submit that Ferguson

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 13

PATENT
Attorney Docket No.: JHU1630

fails to disclose that hypermethylation of CpG islands in the promoter of the cyclin D2 nucleic acids, as compared with the methylation of comparable nucleic acids in normal samples, is indicative of primary breast cancer.

As Ferguson fails to disclose each and every element of claims 1, 25, 26 and 30, as would be required to establish anticipation under 35 U.S.C. § 102(a), Applicants respectfully request reconsideration and withdrawal of the rejection over Ferguson.

B. Applicants respectfully traverse the rejection of claims 1, 25-27, 30, 33 and 34 under 35 U.S.C. § 102(a) as allegedly being anticipated by Esteller et al. (*Cancer Research*, 58:4515-4518; hereinafter "Esteller"). By the present communication, previously presented claims 26-27, and 33-34 have been cancelled without prejudice, rendering the rejection moot as to the subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 25 and 30.

Applicants submit that the invention methods for detecting breast cancer or DCIS in a subject, as defined by amended claims 1, 25, distinguish over the disclosure of Esteller by requiring:

determining the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids isolated from a sample comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject, wherein a state of hypermethylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids as compared with the state of methylation of one or more CpG islands in the promoter of cyclin D2 nucleic acids in comparable samples obtained from normal subjects is indicative of breast cancer or DCIS in the subject.

In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 14

PATENT
Attorney Docket No.: JHU1630

By contrast, Esteller is absolutely silent regarding all elements of the invention methods for detecting primary breast cancer as defined by amended claims 1 and 28. The Examiner asserts that Esteller discloses a method for determining the methylation state of CpG rich region of the GSTP1 gene promoter using methylation specific PCR in samples of breast tumor from a subject and compared to the methylation status of the nucleic acids in normal breast tissue.

However, Applicants respectfully submit that Esteller fails to disclose a method for determining the presence of breast cancer or DCIS in a subject by determining the presence of hypermethylation of CpG islands in the promoter of the cyclin D2 nucleic acids, as compared with the methylation of comparable nucleic acids in normal samples, as is required in the invention methods.

Therefore, Esteller fails to disclose each and every element of claims 1, 25 and 30, as would be required to establish anticipation under 35 U.S.C. § 102(a). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection over Esteller.

In view of the above amendments and remarks, Applicants submit that all rejections of the claims are overcome and Applicants request favorable action on all pending claims. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

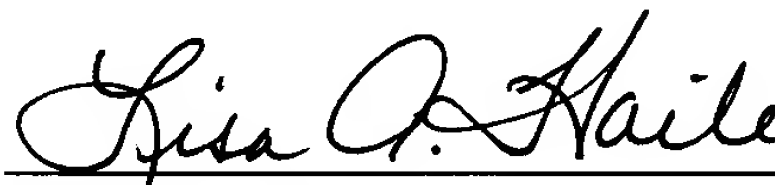
In re Application of:
Sukumar et al.
Application No.: 09/771,357
Filed: January 26, 2001
Page 15

PATENT
Attorney Docket No.: JHU1630

The Commissioner is hereby authorized to charge any fees that may be associated with this Amendment, or credit any overpayment to Deposit Account No. 50-1355.

Respectfully submitted,

Date: October 3, 2003



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Enclosure: Marked up copies of Figures 1-8 (21 sheets)

(SEQ ID NO: 105)



Cyclin D2 promoter, MSP primers
Accn. No. U47284 Promoter region analyzed: -1616 to -1394 bp

1 gagctCGagc caCGccatgc cCGctgcaCG tgcagcttg CGcagcacat cagggCGctg
61 gtctctccc ttctctctgg agtgaaatac accaaagggC GCGtggggg tggggggtga
121 CGggaggaag gaggtgaaga aaCGccacca gatCGtatct cctgtaaaga cagccttgac
181 tcaaggatgC Gttagag CGacCCctgct ggCGgacttc aCGcagtCG
241 gctcccaggg agaaagcctg gcagagtgaag gCGCGaaacC GgagggtCGg CGaggatgCG
301 ggCGaaggac CGagCGtgga ggcctcatgc ctCGgggaa aggaaggggt ggtggtgttt
361 gCGcaggggg agCGagggg agcCGgacct aatccctcac tCGccccctc cccctccCGg
421 gccatttctt agaaagctgc atCGgtgtgg ccaCGctgag gCGgaggaC GgaggCGgct
481 tgtcagcaga tgcaggggCG aggaagCGgg tttttcctgC GtggcCGctg ggCGgggggaa
541 cCGctgggag cctgccccC GgcctgCGgC Ggcccctagac GctgcacCGC GtCGccccac
601 gggccccCGaa gagccccag aaacaCGatg gtttctgctC Gaggatcaca ttctatccct
661 ccagagaagc acccccttc ctctctaata ccaccttc cctccctctt ctctctctgc
721 acacactctg cagggggggg cagaagggaC Gttgttctgg tccctttaat CGgggcttct
781 gaaacagctt CGaagtattc aggaacacag acttcaggga catgacctt atctctgggt
841 atgCGaggtt gctattttct aaatcaccc cctcccttat tttcaccta agggacctat
901 ttctaaattg tctgaggtca cccatcttc agataatcta cctacattc ctggatctta
961 aatacaaggg caggaggatt aggatcCGtt ttgaagaagc caagttgga gggCGtatt
1021 ttggCGtgct acacctacag aatgagtga attagaggc agaaatagga gtCGgtagtt
1081 ttttgtgggt tgcctgtcCG gggcccttg catgcaggct ggatggagg agaggggtg
1141 ggggtggCGg gggacCGCGt ttgaagtgg gtCGggccag ctgctgttct ccttaataac
1201 gagaggggaa aaggaggag gaggggag attgaaagga ggaggggag acCGggaggg
1261 gaggaaggg gagaggaac cagagCGggg aggCGCGggg agaggaggga gagctaaactg
1321 ccagccagc ttgCGtcacC GcttcagagC GgagaagagC Gagcagggga gagCGagacc
1381 agttttaagg ggaggacCGg tgCGagtgag gcagcccCGa ggctctgctC Gcccaccac
1441 caatcctCGc ctcccttctg ctccacctc tctctctgcc ctccctctc cccCGaaaaac
1501 cccctattta gccaaaggaa ggaggtcagg gaaCGctct cccctccct tccaaaaaac
1561 aaaaacagaa aaacctttt ccaggcCGgg gaaagcagga gggagagggg cCGcCGggct
1621 ggcc gag

FIGURE 1A



MSP Unmethylated 223 BP

GT TATGTTATGT TTGTTGTATG

T AAAATCCACC AACACAATCA

Forward UM 22 BP MT 56

Reverse UM 21 BP MT 56

(SEQ ID NO: 21)
(SEQ ID NO: 22)

MSP Methylated 223 BP

TAC TGTGTTATGT TTGTTGTATG

CGT AAAATCCACC AACACAATCA

F M 19 BP MT 58

R M 20 BP MT 56

(SEQ ID NO: 23)
(SEQ ID NO: 24)

FIGURE 1B



(SEQ ID NO: 106)

Twist Promoter: Accn No. AC003986

Promoter Region analyzed: nts -51145 TO -51750

1 cattggactg ggtttccttc cacCGaagag tgaactctg cctctttCGa gcacctcCG
61 aggCGtagtc ctttgatgt tgggagCGt cagactgggt CGttgtagag gggaaaggag
121 ggccagaag ggCGagagag caggCGgga CGcaaatcct cagccccCGC GgCGCGccac
181 Gtcttcagaa aCGccaggac ctCGggctg ggcCGcCGG gtttggcctt tggaactcaa
241 gggttCGtct acctgacct tgggtggctc CGCGgttgac acttttcttg gcatgcccc
301 ccacccCGCG ccacacc ccccgccc cagcaatcca aatCGgccc aCGgacctag
361 agggctcttg ggCGagatga gacatcccc actgtgtaga agctgttgc attgctgtg
421 tcacagcca tCGgatgg gctgccacCG tggccaggac agtctctcC GacCGcttcc
481 tgggctgCGc taggttCGg gggCGctgcc CGcaCGctcC GgCGgggaag gaaatCGccc
541 CGCGccCGC GgaggaaggC GaCGgggagg gaagggggag ggCGgctagg aggCGggtgg
601 aggggcCGc CGccCGggc agtCGctt tgaatggtt gggaggacCGa attgttagac
661 ccCGaggaag gaggtggga CGgggaggg ggactggaaa gCGgaaactt tcctataaaa
721 cttCGaaaag tccctctcc tcaCGtcagg ccaatgacac tgcctcccc aaactttcCG
781 cctgcaCGga ggtataagag cctccaagtc tgcagctctC Gcccaactc cagacacctc
841 gCGggctctg cagcacCGgc accGtttcca ggaggcctgg CGgggtgtgC GtccagcCGt
901 tgggCGcttt ctttttggga cctCGgggcc atccacacCG tccctcccc ctccCGctc
961 cctcccCGcc tccccCGCG GccctcccCG CGgaggtccc tccCGtCGt cctcctgctc
1021 tctcctcCGC GggcCGcatC GccCGggcCG gCGcCGCGC Ggggggaagc tggCGggctg
1081 aggCGccccCG ctcttctct ctgcccCGgg ccCGCGaggc caCGCGtCGc CGctCGagag
1141 atgcagg aCGtgtccag ctCGccagtc tCGcCGgccc aCGacagcct gagcaacagc
1201 gaggaagagc cagacCGgca gcagCGcCG gCGgcaagC GCGggggaCG caagCGgCGC
1261 aCGagcaggC GcaCGgCGgg CGgCGgCGCG gggccCGgCG gagCGggtgg gggCGtCGga
1321 ggCGgCGaCG agcCGggcag ccCGggccag ggcaagCGCG gcaagaagtc tgCGggctgt
1381 ggCGgCGgCG gCGgCGgCGg CGgCGgCGgC Ggcagcagca gCGgCGgCGg gagtCGcag
1441 tcttaCGagg agctgcagac GcagCGggtc atggccaaCG tgCGggagCG ccagCGcacc
1501 cagtCGctga aCGaggCGtt CGcCGGctg CGgaagatca tcccaCGct gccctCGgac

FIGURE 2A



(SEQ ID NO: 106) CONT)

1561 aagctgagca agattcagac cctcaagctg gCGgccaggt acatCGactt cctctaccag
1621 gtcctccaga gCGaCGagct ggactccaag atggcaagct gcagctatgt ggctcaCGag
1681 CGgctcagct aCGccttctC Ggtctggagg atggaggggg cctggtccat gtcCGCGtcc
1741 cac cagg CGgagcccc caccctca gcagggcCGg agaccaggt aaggaccCGCG

FIGURE 2B

Unmethylated 193 BP

tt TGgatggggt tggtatTGT FUM (3) 21 BP AT 58

c ctaaccCAaa CAaccAacc RUM (3) 20 BP AT 60

Methylated 2016 BP

FM (5) 20 BP AT 58

RM (4) 19 BP AT 58

FIGURE 2C



RAR beta promoter, MSP primers

ACCN NO. AF157483

(SEQ ID NO: 91)

Promoter region analyzed: nt -196 to nt -357

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1 gtgacagaag tagtaggaag tgagctgttc agaggcagga gggctctattc ttgccaag
61 gggggaccag aattcccat gCGagctgtt tgaggactgg gatgCGGaga aCGC
121 CGGagg gtttgtctgg gcacCGtCGg ggtaggatcC GgaaCGcatt CGgaaggcctt
181 ttggaagca ttacttggga aggagaactt gggatcttctc tgggaacccc CCGccccCGgc
241 tggattggcC Gaggcaagcct ggaaaatgca attgaaacac agagcaccag ctctgaggaa
301 ctCGtcccaa gccccccatc tccacttctc cccctCGag tgtacaaacc ctgcttCGtc
361 tggcaggaca aatcatcagg gtaccactat ggggtcagCG cctgtgaggg atgtaagggc
421 tttttcCGca gaagtattca gaagaat CG atttacactt gtcacCGaga taagaactgt
481 gttattaata aagtcaccag gaatCGatgc caatactgtC Gactccagaa gtgctttgaa
541 gtgggaatgt ccaaagaatc tgtcaggaaat gacaggaaaca agaaaaagaa ggagacttCG
601 aagcaagaat gcacagagag ctatgaaatg acagctgagt tgggaCGatct cacagagaag
661 atcCGaaaag ctcaccagga aactttccct tcaactctgc agctgggtaa atacaccaCG
721 aattccagtg ctgaccatCG agtcCGactg gacctgggccc tctgggacaa attcagtgaa
781 ctgggccacca agtgcattat taagatCGtg gagtttgcta aaCGtctgcc tggtttcaact
841 ggcttgacca tCGcagacca aattaccctg ctgaaggcCG cctgcctgga catcctgatt
901 cttagaattt gcaccaggta taccaccagaa caagacacca tgactttctc agaCGgcctt
961 accctaaatC Gaactcagat gcacaatgct ggatttggtc ctctgactga ccttgtgttc
1021 acctttgcca accagctcct gcctttggaa atggatgaca cagaaacagg ccttctcagt
1081 gccatctgct taatctgtgg agacCGccag gaccttgagg aacCGacaaa agtagataag
1141 ctacaagaac cattgctgga agcactaaa atttatatca gaaaaagaCG accagcaag
1201 cctcacatgt ttccaaagat cttaatgaaa atcacagatc tcCGtagcat cagtgtctaaa
1261 ggtgcagagC Gtgtaattac cttgaaaatg gaaattcctg gatcaatgcc acctctcatt
1321 caagaaatgc tggagaattc tgaaggacat gaacccttga cccaagttc aagtgggaac
1381 acagcagagc acagtcctag catctcacc agctcagtgg aaaacagtgg ggtcagtcag
1441 tcaccactCG tgcaataaga ca
```

FIGURE 3A



Homo sapiens serine protease-like protease (nes1) mRNA, complete cds ACCESSION

AF024605 (SEQ ID NO: 94)

```
1 accagcgga gaccacaggc agggcagagg cacgtctggg tccctccct ccttcctatc
61 ggcgactccc agatcctggc catgagagct ccgcacctc acctctcgc cgcctctggc
121 gcccgggctc tggcgaagct gctgccgctg gctatgggc aactctggc cgcagaggcg
181 gcgctgctcc ccaaaacga cacgcgcttg gacccgaag cctatgggc cccgtgcgcg
241 cgcggctcgc agccctggca ggtctcgctc ttcaacggcc tctcgttcca ctgcgcgggt
301 gtcctggtag accagagttg ggtgctgacg gccgagcact gcggaacaa gccactgtgg
361 gctcgagtag gggatgatca cctgctgctt cttcagggcg agcagctccg ccggacgact
421 cgctctgttg tccatcccaa gtaccacag ggctcaggcc ccatcctgcc aaggcgaaacg
481 gatgagcacg atctcatgtt gctaaagctg gccaggcccg tagtgccggg gccccgcgtc
541 cgggccctgc agcttcccta ccgctgtgct cagcccgag accagtgcc a ggtgctggc
601 tggggcacca cggccgcccg gagagtgaag tacaacaagg gcctgacctg ctccagcatc
661 actatcctga gccctaaga gtgtgaggtc ttctaccctg gcgtggtcac caacaacatg
721 atatgtgctg gactggaccg gggccaggac ccttgccaga gtgactctgg agggcccttg
781 gtctgtgacg agaccctcca aggcctcctc tcgtggggtg tttaccctg tggctctgcc
841 cagcatccag ctgtctacac ccagatctgc aaatacatgt cctggatcaa taaagtcata
901 cgctccaaact gatccagatg ctacgctcca gctgatccag atgttatgct cctgctgac
961 cagatgcccc gaggtcccat cgtccatcct ctctctccc agtcggctga actctcccct
1021 tgtctgcact gttcaaacct ctgccgccct ccacacctct aaacatctcc cctctcacct
1081 cattccccca cctatcccca ttctctgcct gtactgaagc tgaatgcag gaagtggtagg
1141 caaaggttta ttccagagaa gccaggaaag cggtcatcac ccagcctctg agagcagtta
1201 ctgggggtcac ccaacctgac ttctctgccc actccccgct gtgtgacttt gggcaagcca
1261 agtgccctct ctgaacctca gtttctctcat ctgcaaaatg ggaacaatga cgtgcctacc
1321 tcttagacat gttgtgagga gactatgata taacatgtgt atgtaaatct tcatgtgatt
1381 gtcatgtaag gcttaacaca gtgggtgggt agttctgact aaaggttacc tgttgtcgtg
1441 aaaaaaaaaa aaaa
```

FIGURE 4A



Sequence analyzed: nts +169 to +349
Exon 3 sequence

(SEQ ID NO: 95)

CCGcagaggC GgCGctgctc ccccaaaaCG acaCGCGctt ggacCGGaa GGtgCGC
GCGCGgctCG cagccctggc aggtctCGct CGgc ctctCGttcc actgCGCGgg tgctctgggtg gaccagagtt
gggtgctgaC GgCGctgctc CGGaa GGtgCGC

FIGURE 4B

Unmethylated 128 BP

tTGtagaggT GgTGttgttt

CACACAat aaaaCAaaaa acca

Nes1 FUM 20 BP AT 56

(SEQ ID NO: 77)

Nes1 RUM 22 BP AT 56

(SEQ ID NO: 78)

Methylated 128 BP

CCGcagaggC GgCGctgctc

GCGCGgctCG cagccctggc aggtctCGct

Nes1 FM 20 BP AT 56

(SEQ ID NO: 79)

Nes1 RM 20 BP AT 58

(SEQ ID NO: 80)

FIGURE 4C



HOX A5 Promoter 3' to 5'

AC004080

(SEQ ID NO:96)

16321 accaagagag actgggagag ggCGgcagag aagagagggg ggacCGagag cCGCGtcccc
16381 gCGgtCGCGt ggatttagaa aaaggctggc ttaccatga cttatgtgca gcttgCGcat
16441 ccaggggtag atctggggtt gggCGggCGg CGcCGggctC GgctCGctct gCGcactCGc
16501 ctgctCGctg ctggcagggg CGtcctcctC GgctcCGgaC GCGtgccaa cccctctct
16561 gctgctgatg tgggtgctgc CGgCGtCGgc CGaggCGcCG ctggagttgc ttagggagtt
16621 tttccCGcCG tgggtgctgt CGctgcCGgg CGaggggggc aCGgCGgagc agggcagCGg
16681 atCGggctga ggagagtCG gggaCGtggc CGgctggctg tacctgggct CGgCGggCGc
16741 CGCGctggCG ctggcagCGt agctgCGggc GCGctctcCG gagccaaagt ggcCGgagcc
16801 CGagCGgcCG aCGctgagat ccatgccatt gtagcCGtag cCGtacctgc CGgagtgcat
16861 gctCGcCGag tccctgaatt gctCGctcaC Ggaactatga tctccataat tatgcaactg
16921 gtagtcCGgg ccatttggat agCGacCGca aaatgagttt acaaaataag agctttg
16981 ttttttgata tgtgtgcttg atttgtggct CGCGgtCGtt tgtgCGtcta tagcacctt
17041 gcacaattta tgatgaatta tggaaatgac tgggacatgt acttggttcc ctctaCGta
17101 ggcacccaaa tatggggtac GacttCGaat caCGtgcttt tgttgtccag tCGtaaatcc
17161 tgcctgatga cctctagagg taaactCGtg cactaatagg ggagttgggt ggaggCGagg
17221 ggggtggCGC GCGCGcccCG ggCGCGtgcc CGcCGccagt tgcCGcCGtt cagcCGgact
17281 CGagCGccac cCGctggagg cagggtcat CGcccagctt cCGacCGggg gctgcaaggg
17341 cCGgggtCGa attgaggta cagccatta tggcaaaatt attgcatttc cctCGcagtt
17401 ccattaggat gtaccaattg ttaggcCGtc agctgcCGat CGCGCGcccCG gCGaggatgc
17461 agaggattgg

FIGURE 5A



(Seq ID No 97)

Complement- 5' to 3' Promoter region analyzed: nts -97 to nts -303

ccaatcctct gcatcctCGC CGggCGCGG atCGgcagct gaCGgcctaa caattggtac atcctaattgg
aactgCGagg gaaatgcaat ~~aaatttgaca taattgggcttg taatgctcaat~~ tCGaccccCGg cccttgccagc
ccCGgtCGg aagetggCGG atgagccctg ccCGgcagCGg gttgCGctCG agtcCGgctg aaCGgCGgca
actggCGGCG ggcaCGGCG cCGccacccc.cctCGcctcc acccaactcc cctattagtg
caCGagttta cctctagagg tcatcaggcaggatttaCGa ctggacaaca aaagcaCGtg attCGaagtC
Gtacccata ttgggtgcctaCGtaggaq ggaacccaagt acatgagcca gtcatttcca taattcatca
taaattgtgc aagggtgcta tagaCGcaca aaCGacCGCG agccacaaat caagcacaca
tatcaaaaacaa agct cttatttgt aaac ~~aaatgg ccCGgactac~~
cagttgcata attatggaga tcatagtcc GtgatCGagc aattcaggga ctCGgCGagc atgcactcCG
gcaggtaCGg ctaCGgctac aatggcatgg atctcagCGt CGgcCGctCG ggctcCGgcc actttggtct
CGgagagCGC gccCGcagct aCGctgccag CGccagCGCG gCGccCGcCG agcc ~~_____~~
~~_____~~ caCGtcca CGcactctcc tcagccCGat

FIGURE 5B



UnMethylated 213 BP
tTGgtTGg aagttgggTG FUM 18 BP AT 56 (SEQ ID NO: 71)
gtaTGtg attTGaagtT Gtatt (SEQ ID NO: 98)
aataC AacttCAAat caCAtac RUM 22 BP AT 56 (SEQ ID NO: 72)

FM 18 BP AT 58 (SEQ ID NO: 69)
taCGtg attCGaagtC Gtat (SEQ ID NO: 99)
RM 20 BP AT 56 (SEQ ID NO: 70)
FIGURE 5C

should be
connected in
the seq. ligase

tttgcggggtggtgcggtc



Homo sapiens 14-3-3 sigma protein promoter and gene, complete cds.

ACCESSION No. AF029081

SEQ ID NO: 1024

```
1  ggatcccagc ctgcccctcc acttctctcc caagccaggt cccggcatgg gtgggttatg
61  ctcatgctgg caatacttga aacgggttta ttaatgctgg gtattttgca caattttata
121  gacctctttt ctacatagtc ttttttaaat ggaaggagaa aatgtcagcc acattactgt
181  ctgtgtagtg ccaggagaag ggttatcaga aggcctggtg gtatttaata gtttatcca
241  agagaccttc tggctggaat ggtgagagt gtgtgtgcat gttgtgtgtg gttcatgtgt
301  gccctgtatg aatgtggctg gctcccagat cccctgggct gcccctgcc ccatcccctt
361  tgagtatcag aagcactctg agccaaggga acagggggca cgtgcactgg tcacgagaaa
421  accctgggct cccactgggg ctccagccag cctcctatct ttccttcttc tatggacttc
481  agacagccag tgtctgggga ctctgccact ctaccccag ccctaccac cagccccag
541  gtgaggcttc cagctgggac ctgcccagac aggcctgagcc tgggcgtggg ggggtgggtg
601  atggctctgg ggagcggctg ccctcctaca agccacaccc cctcctctga gctctgaata
661  tgggacccag tgccaggagc tggaagacaa ggtgtttctg ccaaacggga cctccatcca
721  gagaaaagga agaagtgca ggtgggcca agaggcaagt gaaggttggc ctgagctctgg
781  gccggaact cagaggatgt ttctcctctg ctgggagctg tagtttctta tcaaaataga
841  tattgttcca ccatccccct ccttgccctt tcaagtgggc tgaagccttg gaaagtgaca
901  taggaagtcc ccagatcttg ccctctcac tccagaggct agtggtcaca gacagctggg
961  aatggcagcc acagagggtc cctctggaga aacagcttca cccagcctc agggccctgg
1021  gcatcactgc agtggccctg ggaggtgagg aagaagctgg ctagaggagg gggctcccac
1081  ctacctttta ttaagccag tattctttgt tcctgcttgt aataaaactt cagtttataa
1141  gaggttgctt gctttggtt gggttttgtt tgcttttctt ttgctgaggc cccaactggg
1201  agccctctgt tcttcagac aaatttggtt ctttcctggg gagactgtga gaaggcaggc
1261  agcccagtga tctggctaca tttccctca cctggctgga gctctgtccg ctggaggaaag
1321  agcagagagg gctgcggctg agcccccatg ggcacgtgaa aagaggccat cctgtcccct
1381  ctttgtcccc tccaccttc cctgcctcag gggcttgag acccaaat cttcttccct
1441  actgccttcc cactccgac ccaatgagt gccagctaa gaaaatgttt gagacagtag
1501  attccagttt gagagccgga gcttccctgg ctaccacctc caacctgggc accagggccc
1561  agccagacaa ctcataacac tggcccacct ctctgggtatc tccctcagga ggacacctgt
```

FIGURE 6A



(SEQ ID NO: 102 (CON'T))

1621 caggattttg ccattcctg cacagcctga gggagctaa caggcctctt tgcagagggg
1681 tagctggtaa gaccgtttct tcctgtcgg ccagcactgc ccgtccctt ccacacacca
1741 tctcatcctc atcgcatgcc tcgccaaccc catggagccc gtccatctgt ctggtgtgtg
1801 gtgcggtgtg tgtgctgggt ggcaaggcta aaagcccagc ccatttgtgg actgaggaa
1861 cgggatatag ctccagctgg aagaggaggt ggagggtgag gctggggaga ggatggcgaa
1921 cagagcagct cctgcttggg tctgtgctgg tggggtcctg gtatgcaggg gccaccgggc
1981 cctgccctga ggtgcttggg tctgtgctgg tggggtcctg tttctctcac ccgcccgttt
2041 actaacactc ttatgtcctg gctttctgtc ccgctgagc tctctcgggc agaggcaggt
2101 tctctcctgc ttattgcct gctgcctaag ccttgccctt cctctccct cctccaggc
2161 gctgtggcag cactctccc caccaccggg cccctgcagg cggatctcca gtgtgtcgg
2221 ctgctaacc tctctctctt ccttcttgc tctcctgccc gggatctcca gtgtgtcgg
2281 gggcttaagg acctcctgag gaccgctgct ccttgccctt ccaggaatgg cctgggggga
2341 gccaggcacc cggcacctcc acctgcctaa cctgtggccc atctgccacc atctgtgcct
2401 acagggtctg cccccagcc tgcccggcct gtgtgctctc taggacccca tagggggcag
2461 gggctggcct cttggccca ttcccgctcc atgcccggca gagtgtagaa agccataacg
2521 cacgcagcca tcagacaat aatgtgactc taagctgata tgctccctct ctcctccact
2581 gacttcccct tccggattt gtgaggtgtc aagactagga atctggcctt agagcctgcc
2641 cctccacccc ctcatcag catagccat agtcaagccc agcaggttct ctcaaggagct
2701 gtctggggtg ttgatgggtg atgacgtgc tgaacaagt ttggtgactgt tctaagcaca
2761 actggcctga tactgttccc acggcctgtc cactccac ccccaacct caccagagt
2821 aggtaggatg tagggagggt gcgtgccgcc ttgtctctag gactgaggg accaagctag
2881 ccgtgcacag ccccatcac ttcaggggag taaaggaaag agctgagcca aggaaatca
2941 gctgagcca gggctggggg ctgcttgtct gctatcctgt acctttttt ttttaacca
3001 aaataaagat tcccctctc ttgccatacc attggctgtc tgggtggcgc tttactttgg
3061 ggcccaggga tgggacctgc agtgggctg tggaacatat ggctccccct cgctcccagc
3121 tttcttccag ctggccagtg ctgctctgga gattacaag cacaacgaag ccaggaggga
3181 cacaggaaaa gtggctgaca tcctttcac tctgcccctc cagaactctt ggtctcaatt
3241 ccagacacca ccagcctta gctgacctct ggattctgat aggtcccagt gcaggctgag
3301 acagaggggt taactccagt ttgggactgc cataccatg aactgagccc agcccagggt
3361 aacgatactc tggaaacttc tctctccca gttgctgcac tacatcaaga tacacacatg
3421 tgcatacact gtactatggg ctaaaaaat acgtaccgct accgttcagg aagggttgc

FIGURE 6B



(SEQ ID NO: 102 (CONT'))

3481 cgagtcaccg gccatttc tcactttaac ctgtgaggag gatgtgtca gccttttac
3541 agatgagga actgagactc aaggaagaaa caggagctgc ccaaggtcac ccagctggca
3601 aagcagcaaa tccagatcg gaacctgac tctgccccga gctctgagcc atctgcacta
3661 cccaaggaaat gaatacagcg gtggaggagat gagatcttgg agaaacccta aaattagaga
3721 atgtcatagc cagtagaggg cttagagttg atctgggcca gcctccttgt ttactgatg
3781 gagaaattga agccagagg caggaaggga cctgcccag gccttataac agagctggga
3841 tgcagtccca cactctgacc tcattccatt ctctctccat aaattctgca ctgtctctag
3901 actggactgg tttagatgtg ggatactcta aacagcagtg ccttcaagag aaaaagaatc
3961 agaactacga atcacttaaa agtaatgtaa gctactctgg gcacactgcc tatggggtcg
4021 cctgctcca caaggagcca caaaaataat taaaataatt taatatccct tcccaagggt
4081 aaccagtaaa gtaagctctt ggctaggtaa ctggactctt gttcacaaact agccagtggg
4141 aaaagggtgct agagcttctt ctggccacct gtttaatttg atcattccaa gacagaaaca
4201 tttcttagga agttctttct agaactacc tgggtgccct cccactgcta tcagagccct
4261 gtcctctgtc ctcagtggag gtagagagca aatggttgct gctttcttca tcacaaccct
4321 tcaaagccta ttattaccag ctaagaagga ttggttgact atgggccaga gcccctgagc
4381 ctgctggtag aatggatgct gtacaggagg gtggggagggt agcaggcaga atgaggaaag
4441 cccctttgag ctgcaacccc agctcctgtc ctgctgactc agacagctga ctgtggagct
4501 ccatgccctg ccagggccctg ctgccctcctg cccgtctgag ctctgaaact tgggaaatgg
4561 aggccacagag gcaaggagg gtacctgaga caggaaactga gtcaggatca acaggccaga
4621 gcgggcagga ggtatcaggc agctggctc ccagatgcac ccctgagctc cagcagggga
4681 ggagtaggaa tgaaggggct tccttgccct tgctcatggc tatgcgaggg gcgtgaacca
4741 ccaccagggtc ctctggctta agtggcggga agcaaatggt ccctccctgg actcaggctc
4801 caaagtctct gggcctgcct tccaggttcc cagtgtcctg ggatctccag ctttccccag
4861 gacttgggga agccccggct ggatgactag tacaatatgaa ggccccctgag gtccaggac
4921 ctgctgaggt cacaggaata tcctagatca agcttgtcca acccacggcc cacaggctgc
4981 atgtggccca gaatggcttt gaatgcagcc caacacaaat tagtaaaact tcttaaaaca
5041 ttatgagatt tttttgcaaa ttttttttt ttttttagct catcagttat tggtagtggt
5101 ggtatatatt atgtgtggcc caagacaatt ctccaatgt ggcccaggga agccaaaaga
5161 ttggacacgc ctgtcctaga tggagaggaa ggaggcagtg ctgagcacat ctggccattc

FIGURE 6C



SEQ ID NO: 102 (cont.)

5221 atccatctgg agagagaagg ctatgggcaa actgcttcct ctcccctgta gacacccagc
5281 tgggaaggctc tggcctttgg taagtcctgg ctctgggtcc ttctcatatt cacagaaacct
5341 aactctatgt tagtgctttg tagtatatg tgatcataa taaagttgac gggatttttt
5401 cacatgataa taatagttgt catctggccg ggcattggtg cttatgccta taatttcagc
5461 actttggaag gctgaggcag gtggatcact tgggtcagc tgctcgagac cagcctggcc
5521 aacatggtga aaccacatct ctacttaaaa aaaaaaaata taaaaaatt agctgggtgt
5581 ggtggtgcac ccttgtaac gaggttgag ccagctactc gggaggctga ggcaggagaa tcaactgaac
5641 ccaggagggtg gaggttgag tgggtgaga ttgtgccact aactccagc ctgggtgaca
5701 agagcgaaac tccgtctcaa aaaaaagaa aataataata ataatagtg ccatccattc
5761 tactgtgctt tccattaaact cgtgtaatcc tcacaagtcc cattttatag ttacagggaac
5821 tgagggtcac agagcttaaa tcacttgccc aaggccaaa acagctataa gaattacatt
5881 taggcagtct gattccaaag atactagtct attctgtatc tcatagacaa acaatacata
5941 ttcaactttt ttgtgtgtt ttgttttgag acggagtctt gctctgtcac ccaggctgga
6001 gtgcagtggc gccatctcgg ctactcgcaa cgtccgcctc ccgggttcaa gcgattcttc
6061 tgcctcagcc tccgagtag ctgggactac aggcattgtc caccatgccc ggctaatatt
6121 ttgtattttt agtagagaca gggttttcct gggttagcca gaatggtctc gatctcctga
6181 ccttggtgat caccacctc agcctcccaa agtctgaga tgacaggcgt gagccaccgc
6241 gtccgacctt tattcactat ttataaattg gagagaataa gaaatcaaaa agggcccaggt
6301 gtagtgactc acacctgtaa tccagcact ttgggaagcc aaggcaggag gattgcttga
6361 acccagaagt tcgagaccag cctgggcaac atggtgagac cctgtctcta caaaaaatac
6421 aaaaattagc tgggcgttgt ggtgagcacc ttattcttag gaagctgagg caggaggatc
6481 acctgaggcc aaggagggtg agactgcagt gagctgtgat cataccactg tacttcagcc
6541 tggacatcag agtaagaccc tatctctaaa aaggaaattg agaagaaaga aaatcaaaagg
6601 gaagcaaat cactcactct cactacctca agataccctc tagaagttgg tattttagt
6661 tggttcctat tgttttctgt gtcagttctc tgatttgagc aaaatctttg ggacgtcaaa
6721 cttaaaaatcc cctttacttc cttggaaacc ctgtagcatt agcccagaca tgtccctact
6781 cctccttgtg gcaaagagaa ggatctctgc ttgtgtcccc agagtctctg cctaagcctc
6841 cctccaggag ggaagatgag tgttcagaca ctgagtag ctgggggaga cacaggcctg
6901 tgaattatc ctggctcaac tattagggtc gcagaatccc agtgaaggga gccctacctc
6961 tgagcccat ctaagctttg gctatgggtg gggcagataa gcaggaaatcc atccctatag

FIGURE 6D



(SEQ ID NO: 102 (CONT))

7021 gctcaatgcc aacaccctta ggtgaaactc ttgatgaac ttgaggccag ggctccggca
7081 agcagggaag aacgttggc aacagaggctc tccatctctg aggactctgc caggggtcag
7141 agatggggca atggtcaaaa ggaaggaaac ggccaggcac agtggctcat gccataatc
7201 ccagcacttt gggaggctga ggcaggagga tcgcttgagc ccaggagttt gagacctgcc
7261 tgggcaatgt agtgagatct gctctctatt taaaaaaa aaaaaggaaa gaacaagtaa
7321 acttctgaga aacaggctgg gggaggcatc acgtagctgg aattgctgcc ccataaaaca
7381 gaatgggtatg tgtcactgcc acctcccttt ctcagtcctc tctctccca ggttgctagc
7441 gtccccctgg gggatcaaac tggactgctt ccagcctca gacagagagc agtctgagtc
7501 aggcaggaaa gtgggacagc cggggagctg gacccccacc tctgtgagcc ccgctgggtac
7561 ctgatggcat gtggcttggg gagggcaggt gacctggcgt ggagggccag agggtaaatc
7621 ctcaaaacaag tggcaacagg ccaccaactt gaaagggaaa attgtgtagt gatgggaaat
7681 gtgtccaaca aacctactgg gtgactaatt acaaaggctg ggctggagct tcagaggctg
7741 cttgttaaac acttcattaa gcggcactct gaaagctgcc acctgcgcac tctggggagct
7801 cagaggggac cctgaggggg aatgaggcct tccaaacaca gtctggagct accatctca ggtagactga
7861 gaaggagcct ggatctcact tccaaacaca gtctggagct cataggtcag aggcctcaat
7921 gggagaaaag ctaaaggaaag aggtgacaga aaggagtctc agggaaattgg tggctatgtg
7981 actttgagca aatctaccc ctctctgaga cttagtgttc ccatctctat ggtcctgtgt
8041 gtgtcacaga gacatgggtg gattaaatt cgatcgtgat atgaaagtgc ttgggaaact
8101 ccatggccct acctaaacat gattatcct cactgaacc ccctgaaac aagggggaa gttacctggc
8161 aggattagga acccatcct cctgaacctt tatgggctct gtcaggctg aagcagccag
8221 gggctaagc cagtcccttag ccctggaag ggcactgtga aagtggatct gattgagaa
8281 gccgtttcct gatgtgggca gccatgtgat gccagccccg aacaagaggg ggcagcctgg
8341 agcctggaaa ggtgccagtg caggtggggc ccacgcccag atttctcctg ctgactgttc
8401 tgatgattca ccccacatc ccagcctttt tacctttact gcagagccgg aaagggtgtg
8461 gggaagagag gagagggagg caggtcttgg gccctggtcc cgccccctgc tcctccccac
8521 ccttctctgg gcctggccac ccagccaaa ggcaggccaa gagcaggaga gacacagat
8581 ccggcatatgg tcccaggcag cagttagccc gccgcccgc tgtgtgtccc cagagccatg
8641 gagagagcca gtctgatcca gaaggccaag ctggcagagc aggccgaacg ctatgaggac
8701 atggcagcct tcatgaaagg cgccgtggag aagggcgagg agctctcctg cgaagagcga

FIGURE 6E



(SEQ ID NO: 102 (CONT))

8761 aacctgctct cagtagccta taagaactg gtgggcggcc agaggctgc ctggagggtg
8821 ctgtccagta ttgagcagaa aagcaacgag gagggtctcg aggagaagg gcccgagggtg
8881 cgtgagtacc gggagaagg gtagactgag ctccaggcg tgtcgacac cgtgctgggc
8941 ctgctggaca gccacctcat caaggaggcc ggggacggcg agagccgggt ctctacctg
9001 aagatgaagg gtgactacta ccgtacctg gccgaggtg ccaccggtga cgacaagaag
9061 cgcatacttg actcagcccg gtcagcctac caggaggcca tggacatcag caagaaggag
9121 atgccgccc ccaaccccat ccgctgggc ctggccctga actttccgt ctccactac
9181 gagatcgcca acagcccccga ggaggccatc tctctggcca agaccacttt cgacgaggcc
9241 atggctgac tgcacaccct cagcaggac tctacaaag acagaccct catcatgcag
9301 ctgctgcgag acaacctgac actgtggac gccgacaacg ccggggaaga ggggggcgag
9361 gctccccagg agccccagag ctgagtgttg ccgcccaccg cccgccttg cccctccag
9421 tccccaccc tgcgagagg actagtatgg ggtgggaggc ccaccctc tcccctaggc
9481 gctgttcttg ctccaaagg ctccgtggag agggactggc agagctgagg ccacctgggg
9541 ctggggatcc cacttctt cagctgttg agcgaccta accactggtc atgccccac
9601 ccctgctctc cgcaccgct tcctccgac ccaggacca ggctacttct cccctcctct
9661 tgcctccctc ctgcccctgc tgcctctgat cgtaggaatt gaggagtgtc ccgccttgtg
9721 gctgagaact ggacagtggc aggggctgga gatgggtgtg tgtgtgtgtg tgtgtgtgtg
9781 tgtgtgcgcg cgcgccagtg caagaccgag actgaggga agcatgtctg ctgggtgtga
9841 ccatgtttcc tctcaataaa gtccccctgt gacctcctc ctgtctctt tccagttctt
9901 ggcgatgggc tgggagtggg actggaatct gacttagaga ccctgactt ggacctctga
9961 gttaggggcc tgaactccct aggtggctca gtggcccga cgcaagactt tgagtcagg
10021 tgaggccggg gtcc

FIGURE 6F



H.sapiens Wilms tumor (WT1) gene promoter.

ACCESSION No. X74840

(SEQ ID NO: 103)

1 agcttgcagc ccagcccgg gccagccagg tacaggaggc cggactgcaa ccggttgctt
61 ccctcccgtc gcgcctggcc gtcccacgtc gcgcgctcgc tgctgcctcc tggcgccccct
121 gggattttat acgcacctct ttcccaatag atactgactc ccttagaaga tccaaaaacc aaaccaaaac
181 taggggttgt accccctacc cgccccaaac acctgctctg gggcgcgggg gctgccaaac agagactaga
241 cgaaggaggt cagatttagc gaantcttcg agctcccaaa gattcgaaca ctaactcgcg
301 cccgtgggcc gatggaggtt ctccctactc cactccttg cactcctaac tggcttccgc
361 ctccgtgtca atcactgagc aaccagaatg gtatcctcga ccagggccac aggcagtgc
421 cggcggagtg gctccaggag ttaccgcgtc ctgccgggct tcgtatccaa accctccccct
481 tcacccctcc caagggtttt agatatctct cactggaaag ggaaactaac agtaaacaaac tactagccga
541 ggcgtttgcc tagaagaatt aaccgcttcc gcctggcgca aacctcacca actcccgcc acccaactga gggacgttcg
601 tcgaaatacg cccggcttat aacctcctc cactggaaag ggaaactaac agtaaacaaac tactagccga
721 ctttcagtcc cgacctcttg aacctcctc gacctgcca aacctcacca actcccgcc acccaactga gggacgttcg
841 tcatggccac tccctacctg agcctacctg accctgccc accctgccc gggccacctc ttccccagt gacccaaga
901 caagggtata cgcttctttg aagcttgact aagcttctc gagtctcttc tgcgctttcc tgaagtccc
961 gccctcttgg agcctacctg accctgccc accctgccc gacctgac caaaccactc tttagatta acaaccccat
1021 ctctactccc accgcattcg accctgccc accctgccc tggcgaaggc caagaagggg aggtgtgtgc
1081 gtgagacgag gctcccacac tggcgaaggc gcgtgttggg ttgaagagga ggggtgtctcc gagagggacg
1141 cacaccggcc agctgagagc gcgtgttggg tggcgaaggc caagaagggg aggtgtgtgc
1201 caccaccggc agctgagagc gcgtgttggg tggcgaaggc caagaagggg aggtgtgtgc
1261 ctccctcgga cccgccctca cccagctgc gagggcgccc gagggcgccc ccaaggagca gcgcgcgctg
1321 cctggccggg ctggggctgc tgagtgaatg gagcggccga gcctcctggc tcctcctctt
1381 cccgcgccg ccggcccctc ttatttgagc ttggggaagc tgagggcagc caggcagctg

FIGURE 7A



(SEQ ID NO: 103 (CONT))

1441 gggtaaggag ttcaaggcag cgccacaccc cgggggctct ccgcaacccg accgcctgtc
1501 cgctccccc cttcccgccc tccctcccac ctactcattc acccaaccac ccaccagag
1561 ccgggacggc agcccaggcg cccgggcccc gccgtctcct cgccgcgac ctggacttcc
1621 tcttgctgca ggaccgggct tccacgtgtg tcccggagcc ggcgtctcag cacacgctcc
1681 gctccgggcc tgggtgccta cagcagccag agcagcaggg agtccgggac ccgggcggca
1741 tctgggccaa gtagggccc gccgaggcca gcgctgaacg tctccagggc cggaggagcc
1801 gcggggcgct cggtcttgag cctcagcaaa tgggctccga cgtgcgggac ctgaacgcgc
1861 tgctgcccg cgccccctcc ctgggtggcg gcggcggtg tgccctgcct gtgagcggcg
1921 cggcgcagtg ggcgcggtg ctggactttg cgcccccggg cgcttcggct tacgggtcgt
1981 tgggcggccc cgcccgcca ccggctccgc cgccaccccc gccgcggccg cctcactcct
2041 tcatcaaca ggagccgagc tggggcgggc cgagaccgca cgaggagcag tgcctgagcg
2101 ccttactgt ccacttttcc ggccagttca ctggcacagc cgagccctgt cgctacgggc
2161 ccttcggtcc tcctccgccc agccaggcgt catccggcca ggccaggatg ttccctaacg
2221 cgccctacct gccagctgc ctcgagagcc agcccgtat tcgcaatcag ggtaagtagg
2281 ccgggggagcg ccccta

FIGURE 7B

Estrogen Receptor (ER): Homo sapiens estrogen receptor beta gene, promoter region
and partial cds
Accession Number AF191544 (SEQ ID NO: 184)

1 actatagggc aCGCGtggtc GaCGggccCGg gctgggtattg atagatgcat tttcttcacc
61 ctcacctatc tttttctgcc tggtggtta tggtgaaat tccttcatga CGgttttccat
121 ttccagagat atcttggttaa caagtatata ccaccaaatg aagctgattt tttttttttt
181 ttttttttga gacagagtct CGctctgtCG ccaggctgg aatgcagtgg CGCGatcttg
241 gctcactgca acctcCGcct ccctgttca agCGattctc ctgcctcagc ctctgagta
301 gctgggatta ctggcatgtg ccacCGCGtc cagccaattt ttgtatttt agtagagaCG
361 aggtttcacc atgttggtca ggctggctc aaactcctga cctCGtgatc cacctgcctc
421 ggcctcccaa agtgctgaga ttataggtgt gagccaccat gcctggccat gaagctgatt
481 tttttaaac atcatttaac atttctcca taagggtgga aggaggaaga gcatatgggg
541 actgggtact ttgagagacc ccaggacagg agacaggag gctgagattg gcatgttctc
601 tgctgcagtt atttgccagc Gacacactct ttcCGtccea actaaactct ctgcctcaag
661 gacagggaga ctctgcctt caacctgaga gaaaccaggaa ctctcagctt taatgaaat
721 tggacttagg gtggggcagt ggagactttt cacagctatt gtttagctga tgaagcagat
781 gcttctccat ctttgagcc tgtcttcatt acctgtggac ctcatcttta tcaaccaga
841 gcacacttgC Gtctctctat tttggctaaa caccaaacag ctgaggctgg tactgtaaaa
901 ctttccctcc aaatgcccc cctCGctctc ctctattaga gatctggatc acaaccctca
961 aaaaccatgt cccttatgcc acctgagtag atggtttgat gattaattag gcacagatgt
1021 gacactgggg ggtctcaca atggcctgtg ggtcacatgc tactttcctt ttcattttca
1081 tcagcaacag ctgccttaaa gccagttaag actgtggtcc tagtctCGca ccctggggt
1141 cctgctgggg tgggtgaggg gaacaccca ttaagctggg ggaactgggg ctgccaccag
1201 ggggCGCGag gggccttCGc CGgagaagag ggtgggcag gtgcctccag CGgagaaggg
1261 CGCGtggtgC Ggaggcacag gtctcccCGg tgccactca agtgagttCG aggaagtacc
1321 tgggatcttt gatctaaCGC Gaaaggcctt ccagtgacc tcttgagggc tgagaaccca
1381 ctccctccac ctctagtcca CGgctttgcc actccagggc CGagggttaC Gtttgctgct
1441 ggggatttga caaacccaaa gcctctctgg ttccaccact ggctccttag aatcagacat
1501 ctgttcttgaa tgacacttat gtgagtcagg ggtgaggac GtgatcctCG aagtgtggtc
1561 ccagactgg ctgtatcagt gtCGgcatcc ccaggacct ggttggaat gcatattctc
1621 aggccctact ccagacctct taaatctgag actgggggtg CGgggagCGc catctgtgCG

FIGURE 8A



1681 ccactatcct tgtgggtgga ccaggagtCG gttCGagggt gctccactt agaggtcaCG
1741 CGCGGCGTCCG ggcgttcctg agacCGTCCG gctccctggc tCGgtcaCGt gggctcaggc
1801 actactccc tctaccctcc tctCGgtctt taaaaggaag aagggttta tCGttaagtC
1861 Gcttgtgatc ttttcagttt ctccagctgc tggcttttg gacacccact ccccCGccag
1921 gaggcagttg caagCGCGga ggcgtCGga aataactgg tcttgaaact tgcaggggCGa
1981 ggcgtCGga tg ggcCGgggag ggaccaccCG agctgCGaCG ggcctctgggg
2041 ctgCGgggca ggcgtggCGc CGgagcctg agctgcaggga ggtgCGctCG cttcctcaa
2101 caggtggCGg CGgggCGCGc GcCGggagac ccccctaata ggcgtgCGga
2161 attttagag aaggcaaggc CGgtgtgttt atctgcaagc cattatactt gccaCGaat
2221 ctttgagaac attataatga cctttgtgcc tcttcttga aggtgttttc tcagctgtta
2281 tctcaagac gatataaa aaactcacca tctagcctta attctccttc ctcctacaac
2341 tgcagtcaat ccattctacc cctggagcaC Ggctccatat acataccttc ctcctatgta
2401 gacagccacc atgaatatcc agccatgaca ttctatagcc ctgctgtgat gaattacagc
2461 attcccagca atgtcactaa cttgggaagggt gggcc

FIGURE 8B

Unmethylated 288 BP

G ggTGtttttg agatTGtTGg

FUM 21 BP AT 60

(SEQ ID NO: 85)

TG agttgTGaTG ggtttttg

(SEQ ID NO: 86)

ccaaaacc CATCAcaact CA

RUM 20 BP AT 58

(SEQ ID NO: 87)

Unmethylated 124 BP

FM 18 BP AT 60

(SEQ ID NO: 88)

CGggaaaag taCGtgttCG t

(SEQ ID NO: 89)

RM 20 BP AT 60

(SEQ ID NO: 90)

FIGURE 8C